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Cloning and characterization of the repressor gene of the Staphylococcus aureus lactose operon.
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JOURNAL OF BACTERIOLOGY, Dec. 1987, p. 5459–5465
0021-9193/87/125459-07$02.00/0

Vol. 169, No. 12

Cloning and Characterization of the Repressor Gene of the *Staphylococcus aureus* Lactose Operon

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Received 7 July 1987/Accepted 3 September 1987

The genes responsible for utilization of lactose in *Staphylococcus aureus* are organized as an inducible operon, with galactose 6-phosphate being the intracellular inducer. To clone the repressor gene of this operon, we constructed an integration vehicle carrying 1.9 kilobases (kb) of DNA sequences from a region upstream of the structural genes of the operon. Through integration and subsequent rescue of this plasmid, we were able to clone approximately 7 kb of staphylococcal chromosomal DNA. We have shown that the plasmid insert complemented *lac* constitutive mutants. This repressor activity was localized to a 1.8-kb DNA fragment and, through maxicell analysis, was shown to correlate with the presence of a polypeptide with an apparent molecular weight of 32,000. Furthermore, a region between the repressor gene and the other genes of the operon was identified which, when carried on multicopy plasmids, resulted in expression of the operon in the absence of any exogenous induction. This region may represent an operator-type element capable of titrating repressor molecules away from chromosomal operator, allowing transcription of the operon in the absence of induction.

Metabolism of lactose by *Staphylococcus aureus* is initiated with the uptake of the disaccharide by the phosphoenolpyruvate sugar:phosphotransferase system (20, 28–30). The intracellular lactose-phosphate is then cleaved into glucose and galactose 6-phosphate by the enzyme phospho-β-galactosidase (7, 20, 28). The two β-galactoside-specific components of the phosphotransferase system, enzyme II^{lac} and factor III^{lac}, as well as phospho-β-galactosidase, compose part of a lactose-inducible operon for which galactose 6-phosphate is the actual intracellular inducer (20). Our laboratory has reported the molecular cloning of *lacG*, the gene for phospho-β-galactosidase (7), and the genes for factor III^{lac} (*lacF*) and enzyme II^{lac} (*lacE*) have also been identified (F. Breidt, W. Hengstenberg, U. Finkeldei, and G. C. Stewart, J. Biol. Chem., in press). The genetic arrangement of these genes is as follows: 5′-*lacF-lacE-lacG*-3′.

The expression of the *lac* genes has been shown to be induced with the addition of lactose or galactose to the culture medium. The system is also subject to catabolite repression, although cyclic AMP, at least in physiologically significant concentrations, has not been detected in *S. aureus* (6; unpublished data).

The lactose operon appears to be analogous to other catabolic operons by being negatively controlled. The presence of a repressor molecule for the operon has been alluded to by other investigators (17, 20). Mutations resulting in a lactose constitutive phenotype have been shown to be tightly linked to the *lac* structural genes (7, 20). The purpose of this communication is to report the molecular cloning of the repressor gene (*lacR*) of the staphylococcal *lac* operon in *Escherichia coli*, its localization on the *S. aureus* DNA, and analysis of its gene product as synthesized in *E. coli* maxicells. Furthermore, a region of the chromosome between the *lac* structural genes and *lacR* has been identified which, when carried on a multicopy-number plasmid, results in expression of the operon in the absence of any exogenous inducer. Thus, this locus has characteristics consistent with its being an operator-type element with an affinity for repressor.

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MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* strains LE392, HB101 (16), and JM83 (18) were used as recipients in the cloning experiments. For the lambda phage promoter expression experiment, the recipient strain was *E. coli* N4830 [*sup*^{0} *his ilv* Δ(*chlD-pgl*) (λ Bam N^+ cI857 *H1*)] which carries the temperature-sensitive lambda repressor. Both the pPL-lambda expression vehicle and N4830 recipient strain were purchased from Pharmacia. For maxicell analysis, *E. coli* CSR603 (26) was used. *S. aureus* strains were KUS74, a transposon Tn*551*-derived Lac-constitutive mutant (7), KUS103, and KUS104 (two independently isolated, ethyl methanesulfonate [EMS]-derived Lac-constitutive mutants). These strains are derivatives of RN450, the 8325-4 strain of Novick and Richmond (22). *S. aureus* RN4220 (14) was the recipient strain in all protoplast transformation experiments. Plasmids used in construction of pBO3 and other cloning experiments were pDH5060 (10, 23), pE194 (12), pBR322, pBR327 (16), pMH109 (13), and pMK4 (32).

Media and reagents. L broth with and without 1.5% agar was used for *E. coli* cultivation (19). *S. aureus* cultures were grown either in L broth or in tryptic soy broth or on tryptic soy agar (TSA) (Difco Laboratories).

Other materials were obtained from the following sources: antibiotics, lysozyme, lysostaphin, *o*-nitrophenyl-β-D-galactopyranoside (ONPG), and ONPG-phosphate, Sigma Chemical Co.; bacterial alkaline phosphatase, restriction endonucleases, and T4 DNA ligase, Bethesda Research Laboratories, Inc., and Pharmacia, Inc.; L-[^{35}S]methionine and [α-^{32}P]dATP, New England Nuclear Corp.

Plasmid and chromosomal DNA isolation. Plasmids were routinely isolated from 100-ml overnight cultures of *E. coli* by the alkaline lysate method of Birnboim and Doly (1). Plasmid DNA was further purified by treatment with guanidine hydrochloride as described by Dyer and Iandolo (9). *S. aureus* chromosomal DNA was isolated by the

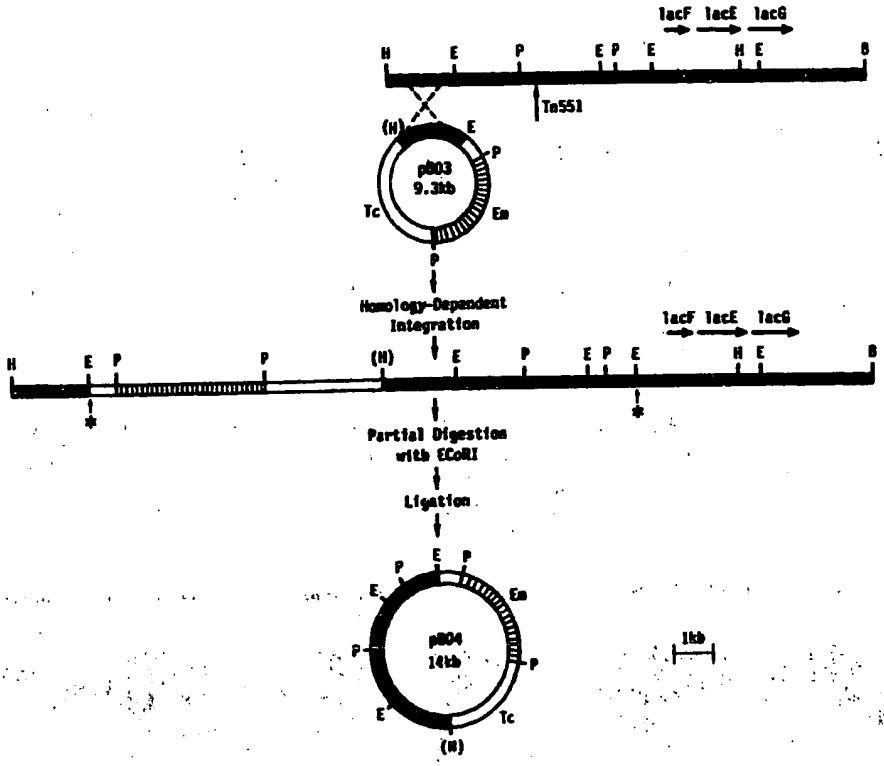


FIG. 1. Isolation of pBO4 through integration and rescue of pBO3. Lac$^+$ strain RN4220 was used. The top of the figure shows the 1.9-kb *Eco*RI-*Hin*dIII fragment on pBO3 which provided the homology required for integration of the plasmid into sequences upstream of the *lac* operon. The site of insertion of Tn*551* in strain KUS74 is indicated with an arrow. The integrated form of pBO3, as well as the *Eco*RI recognition sites (marked with a star) which delimit pBO4, are shown in the center. The bottom depicts pBO4. Heavy black bars are from the *S. aureus* chromosome. White bars represent sequences from *E. coli* plasmids pBR327 and pDH5060, and hatched areas indicate pE194 sequences. Abbreviations: Em, erythromycin resistance gene; Tc, tetracycline resistance gene; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; (H), *Hin*dIII end which was lost when the fragment was made blunt-ended through the use of the Klenow fragment of DNA polymerase I.

method of Dyer and Iandolo (9), followed by CsCl-ethidium bromide density equilibrium centrifugation.

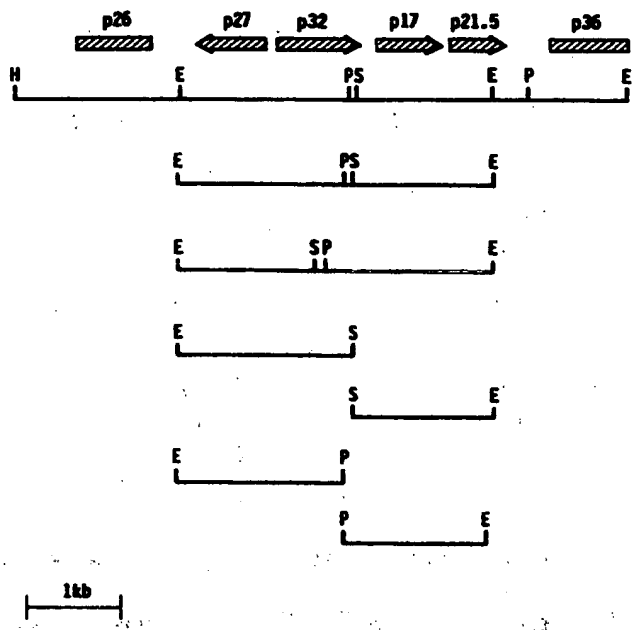
For rapid screening of plasmids, 5-ml overnight cultures of either *E. coli* or *S. aureus* were lysed by the rapid boiling method of Holmes and Quigley (11), except that 10 μg of lysostaphin per ml was used in place of lysozyme when screening *S. aureus* cultures.

Genetic techniques. Introduction of plasmids by transformation into *E. coli* was done as described by Dagert and Ehrlich (8). For introduction of plasmids into *S. aureus*, all plasmids were first introduced into the restriction-minus strain RN4220 (14). This was accomplished by protoplast transformation essentially as described by Murphy et al. (21). *S. aureus* phage 80α was then used to transduce the plasmids out of RN4220 into the other *S. aureus* strains used in this study by the method of Rubin and Rosenblum (25).

Phospho-β-galactosidase assays. Overnight cultures of *S. aureus* were used to inoculate 10 ml of L broth to an A_{540} of 0.05 in nephlometer flasks. In some cultures, 1% (wt/vol) galactose was included to induce the *lac* system. The cells were grown to the mid-log phase of growth (A_{540}, 0.5) and harvested by centrifugation (3,000 × *g*; 10 min). The cell pellets were washed with 5 ml of saline and then suspended in 3 ml of saline. Potassium phosphate buffer (pH 7.5) was then added to a final concentration of 4 mM, and after equilibration at 37°C, an aqueous solution of ONPG was added to a final concentration of 5 mM. The reaction tubes were then incubated in a 37°C water bath for 30 min, and the absorbance at 420 nm was recorded. The amount of activity of phospho-β-galactosidase was expressed as nanomoles of ONP released per milligram of cells (dry weight) per minute.

Expression of the repressor gene product. A 3.2-kilobase (kb) *Eco*RI fragment displaying repressor activity was made blunt-ended by treatment with the Klenow fragment of *E. coli* DNA polymerase I as described by Maniatis et al. (16) and cloned in both orientations in the *Hpa*I site of the expression vehicle, pPL-lambda, which is essentially plasmid pKC30 described by Shimatake and Rosenberg (27). The recombinant plasmids were introduced into *E. coli* N4830. The cells carrying the plasmids were then grown to an A_{600} of 0.5 at 32°C, at which time they were transferred to a shaker waterbath at 45°C to inactivate the lambda repressor. Cells were maintained at high temperature for 60 min and then harvested by centrifugation (3,000 × *g*, 10 min). The cell pellets were each then resuspended in 200 μl of the lysis buffer (2% sodium dodecyl sulfate [SDS], 5% β-mercaptoethanol, 10% glycerol, 50 mM Tris hydrochloride, pH 7.5, and 0.002% bromophenol blue) and boiled for 3 min; 50 μl of each sample was then electrophoresed on a 16% SDS-polyacrylamide gel supported with Gelbond (FMC Corporation), and the protein bands were visualized by staining with Coomassie blue (15).

***E. coli* maxicell analysis.** Plasmids were introduced into *E. coli* maxicell strain CSR603 by competent-cell transformation. Proteins encoded by the resident plasmid in each cell were then labeled with [^{35}S]methionine by the method of



PLASMID	P-β-Gal ACTIVITY[a] N	I	PROTEIN SIZES (kdal)
pBO4	0.00	0.45	26, 27, 32, 17, 21.5, 36
pBO694	0.41	0.2	27, 32, 17, 21.5
pBO695	0.17	0.1	27, 32, 17, 20
pBO696	0.195	0.8	33, 17, 20
pBO697	5.9	5.10	17, 20
pBO732	0.194	7.23	27
pBO733	4.94	8.5	17, 20
pMK4	0.2	6.6	—

FIG. 2. Physical map of pBO4 and its various subclones. All of the staphylococcal DNA inserts (except pBO4) were subcloned into pMK4. Plasmids pBO694 and pBO695 represent the same fragment cloned in opposite orientations. Phospho-β-galactosidase activity of RN4220 cells carrying these plasmids including pMK4 (the vector) is shown in the presence (I) and absence (N) of 1% galactose as the inducer of the operon. The sizes of the proteins encoded in the region, as well as the direction of transcription of their genes (when known), are also indicated. See text for definition of phospho-β-galactosidase (P-β-Gal) activity. Restriction site abbreviations are given in Fig. 1, plus S, *Sal*I. kdal, Kilodaltons.

Sancar et al. (26). Total cell proteins were electrophoresed in a 16% SDS-polyacrylamide gel. The radiolabeled proteins were visualized by autoradiography with Coronex-7 X-ray film (Du Pont).

RESULTS

Construction of pBO3. KUS74 is a Tn*551*-generated lactose constitutive mutant (7). The insertion site of the transposon was thought to identify the *lacR* locus, a negative regulator of the *lac* operon. To clone this repressor gene from a transposon-free strain, we first set out to construct an integration vehicle. This plasmid, designated pBO3, carries a 1.9-kb *Eco*RI-*Hin*dIII fragment of staphylococcal DNA from a region approximately 7 kb upstream of the phospho-β-galactosidase gene. The presence of such a fragment on pBO3 would provide the homology needed for the site-specific integration of the plasmid into the staphylococcal chromosome (Fig. 1). pBO3 carries the origin of replication and tetracycline resistance determinant cloned from the *E. coli* plasmids pDH5060 (10, 23) and pBR327 (16). pBO3 also contains temperature-sensitive gram-positive replication functions plus an inducible erythromycin resistance determinant, both cloned from the staphylococcal plasmid pE194 (12).

Integration and rescue of pBO3. pBO3 was introduced into *S. aureus* RN4220 by protoplast transformation, and plasmid-containing cells were selected on regeneration agar (31) with erythromycin (5 μg/ml) at the permissive temperature of 32°C. The presence of pBO3 in erythromycin-resistant colonies was confirmed by minilysate screening. The cultures carrying pBO3 were then streaked onto erythromycin-containing TSA plates and incubated at the nonpermissive temperature of 45°C. The cells were subcultured to fresh medium every 24 h for 3 days. After three passages at 45°C, cells were once again screened for plasmid presence, and chromosomal DNA was isolated from cultures in which no extrachromosomal plasmid DNA was detected. A variety of restriction endonucleases were then used to digest the chromosomal DNA. The digestion products were treated with T4 DNA ligase in dilute DNA concentrations to maximize intramolecular ligation and therefore circularization of each fragment. The ligation mixture was then used to transform *E. coli* HB101, with selection on L-agar plates containing tetracycline (20 μg/ml).

A 14-kb plasmid, pBO4, was isolated from a partial digest of chromosomal DNA by using *Eco*RI endonuclease. Physical mapping of pBO4 revealed that it contained approximately 7 kb of the staphylococcal chromosome encompassing the region upstream of the structural genes of the *lac* operon (Fig. 1).

Complementation of Lac-constitutive mutants. Insertion of transposon Tn*551* in the region upstream of the *lac* genes conferred a Lac-constitutive phenotype to KUS74 (7). To determine whether pBO4 could complement constitutive mutants of the *lac* operon in *trans*, the plasmid was introduced into two EMS-generated *lac*-constitutive mutants. Also subclones of pBO4 (Fig. 2) designated pBO649 (a 3.2-kb *Eco*RI fragment from pBO4 cloned in pMH109 [13]), pBO696 (a 1.8-kb *Eco*RI-*Sal*I subclone in pMK4 [32]), and pBO697 (a 1.7-kb *Sal*I-*Eco*RI subclone in pMK4) were introduced into the two EMS mutants and also into KUS74. Phospho-β-galactosidase assays were then performed on these cultures as a measure of the activity of the operon. Results of these enzyme assays are shown in Table 1. The presence of the cloned upstream region of the *lac* operon on the multicopy plasmids pBO4 and pBO649 converted the cells from a constitutive phenotype to a noninducible phe-

TABLE 1. Complementation of Lac constitutive mutations

Host	Plasmid	Induction[a]	Phospho-β-galactosidase activity[b]
RN450		−	0.2
		+	6.1
KUS74		−	3.5
		+	9.1
		G	1.5
	pBO649	−	1.6
	pBO649	+	3.7
	pBO696	−	0.6
	pBO697	−	6.6
KUS103		−	5.8
		+	10.1
		G	1.3
	pBO4	−	0.6
	pBO4	+	1.0
	pBO649	−	0.5
	pBO649	+	1.9
	pBO696	−	<0.1
	pBO697	−	8.2
KUS104		−	9.4
		+	21.3
		G	2.4
	pBO4	−	0.3
	pBO4	+	2.2
	pBO649	−	0.5
	pBO649	+	1.6
	pBO696	−	<0.1
	pBO697	−	8.0

[a] +, Culture induced by addition of 1% galactose; −, no induction; G, glucose added to 1%.
[b] Expressed as nanomoles of ONP released per minute per milligram of cells (dry weight).

notype. This complementing activity associated with pBO649 was further localized to the left half of the insert fragment, as indicated by the repressor activity observed with pBO696 but not with pBO697. The presence of pBO4 or pBO649 also rendered wild-type cells noninducible, a feature exploited in the deletion analysis (see below). The plasmids pBO649 and pBO696 corrected the constitutive phenotype of KUS74, the Tn*551* insertion mutant. These complementing effects, along with that of pBO4, were *trans* effects. Curing of the plasmids restored the constitutive phenotype (data not shown). Also shown in Table 1, addition of glucose to the culture medium resulted in repression of *lac* gene expression. With the constitutive mutants, the addition of glucose lowered phospho-β-galactosidase activity to the basal level seen with catabolite-repressed wild-type cells. Thus, catabolite repression exerts its effects on the *lac* operon directly and is not simply an indirect effect brought about by the loss of induction resulting from the inducer expulsion phenomenon (24).

To localize *lacR*, we constructed a number of subclones of pBO4. All of these constructs were made by using plasmid pMK4 (32) as the vector, thus keeping the copy numbers approximately the same. Each of the subclones was subsequently protoplast transformed into the *lac* wild-type strain, RN4220, and phospho-β-galactosidase assays were performed on each clone in the presence and absence of 1% (wt/vol) galactose as the inducer of the operon. The results of such assays and the physical maps of the subclones are shown in Fig. 2. The repressor activity was localized to a 1.8-kb *Eco*RI-*Sal*I DNA fragment carried on plasmid pBO696 (the same DNA fragment which complemented the Lac constitutive mutants). The *lacR* gene was found to span the *Pst*I site of the fragment, positioned approximately 50 base pairs upstream of the *Sal*I recognition sequence.

An interesting finding from this study was the observation that some of these subclones carrying DNA fragments from regions downstream of the *lacR* gene conferred a Lac-constitutive phenotype on the cells which harbored these sequences on multicopy plasmids. This is evident from the level of phospho-β-galactosidase activity in the absence of induction in cells carrying pBO697 or pBO733 (Fig. 2). An increase in the expression of the operon suggested the presence of repressor-titrating regions on these multicopy plasmids. By binding the repressor, pBO697 and pBO733 may free the chromosomal operator from its negative controller and consequently result in expression of the operon under noninducing conditions.

Identification of the *lacR* gene product. *E. coli* maxicell strain CSR603 was transformed with the same pMK4-based plasmid constructs used for the above enzymatic assays (Fig. 4) as well as pBO4 (Fig. 3). The proteins encoded by each resident plasmid were then labeled with [^{35}S]methionine by the method of Sancar et al. (26). These cells were then lysed, their total proteins were electrophoresed on an SDS-polyacrylamide gel, and the gel was autoradiographed. A total of six polypeptides were specified by the staphylococcal insert of pBO4. Of these, a polypeptide with an apparent molecular weight of 32,000 correlated with the repressor activity. This protein was absent with clones whose inserts were interrupted at the *Pst*I site (Fig. 4, lanes 2 and 3), which is also consistent with this polypeptide being

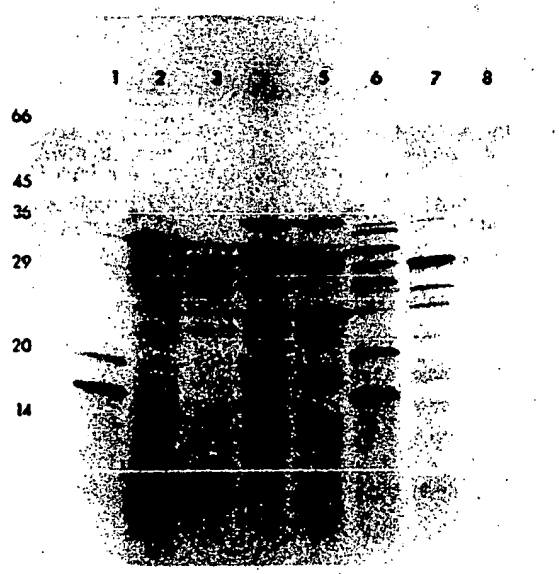


FIG. 3. Maxicell analysis of the proteins encoded in the *lac* region. Plasmid-encoded proteins were selectively labeled with [^{35}S]methionine, electrophoresed on a 16% polyacrylamide gel, and autoradiographed. The plasmids are described in Fig. 2. Shown are lysates of cells carrying the indicated plasmids. Lanes: 1, pBO697; 2, pBO696; 3, pMK4; 4, pBO649; 5, pMH109-5 (a chloramphenicol acetyltransferase-producing clone [13] used to indicate position of this protein); 6, pBO4; 7, pBO3; 8, CSR603 host strain without any plasmid. The numbers on the left designate the positions of the molecular size markers (in kilodaltons).

the *lacR* gene product (see Discussion). *lacR* may additionally cross the *SalI* site. The observation supporting this assumption is that plasmid pBO696 (an *Eco*RI-*Sal*I insert in pMK4), although retaining repressor activity, encoded a protein larger than 32,000 daltons. This larger (approximately 33,000-dalton) protein is most likely a fusion product of the vector sequences continuous with the *Sal*I site (Fig. 3 and 4, lanes 2 and 4, respectively). Furthermore, the gene for the 21.5 kilodalton protein spans the *Eco*RI site, resulting in different-sized products depending on the orientation of the fragment encoding it (compare lanes 3, 5, and 6, Fig. 4). The locations of the polypeptide-encoding sequences are shown in Fig. 2. At this time, we are not able to assign any functions to the other polypeptides.

Promoter activity. The same 3.2-kb *Eco*RI fragment present on pBO694 and pBO695 was cloned in the promoter-cloning vehicle pMH109 (13), and the strength of promoters directing the promoterless chloroamphenicol acetyltransferase gene of pMH109 from both orientations of the *Eco*RI fragment was determined as described (13). Promoter activity was detected in *E. coli* with the *Eco*RI fragment in either orientation. However, the activity was found to be approximately twofold higher with the fragment cloned in the direction opposite to that of phospho-β-galactosidase transcription (4.83 versus 2.17 nmol of chloroamphenicol acetylated per min per mg of cells [dry weight]).

Orientation of repressor gene transcription. As strong promoter activity in the repressor region was detected only in the orientation opposite to that of transcription of *lac* structural genes, it was of interest to determine the direction of transcription of *lacR* relative to that of *lac* structural genes, for the detected promoter activity may have resulted from other gene transcripts. The 3.2-kb *Eco*RI fragment carrying *lacR* was cloned in both orientations into plasmid pPL-lambda so that its transcription was placed under the control of the strong left promoter of bacteriophage lambda

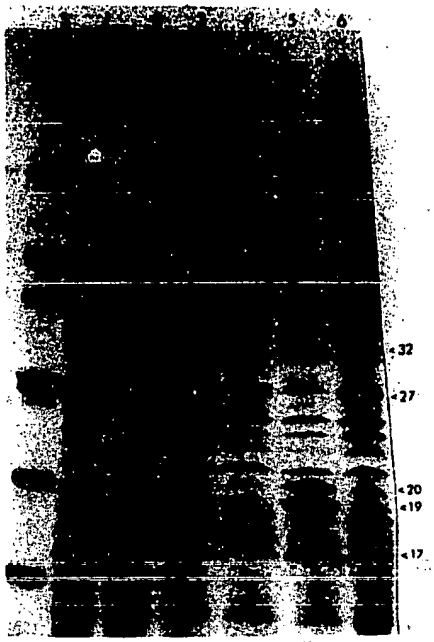


FIG. 5. Determination of the direction of the transcription of *lacR*. Expression vehicle pPL-lambda was used to clone the 3.2-kb *Eco*RI fragment with repressor activity (the insert from pBO649). The proteins encoded on this fragment were then overproduced in *E. coli* N4830 with the fragment in both orientations. Shown is a Coomassie blue-stained 16% polyacrylamide gel. Lane S contains molecular weight markers. Lanes 1 and 6, N4830 host strain without any plasmid; lanes 2 and 3, the *Eco*RI fragment cloned in the orientation opposite to that of *lac* structural genes; lanes 4 and 5, the *Eco*RI fragment cloned in the same orientation as the *lac* structural genes. Cells in lanes 1, 2, and 4 were grown at 32°C, whereas those in lanes 3, 5, and 6 were induced by growth at 45°C. The arrowheads signify the positions of thermally induced, plasmid-encoded proteins. The sizes of the proteins (in kilodaltons) are indicated to the right.

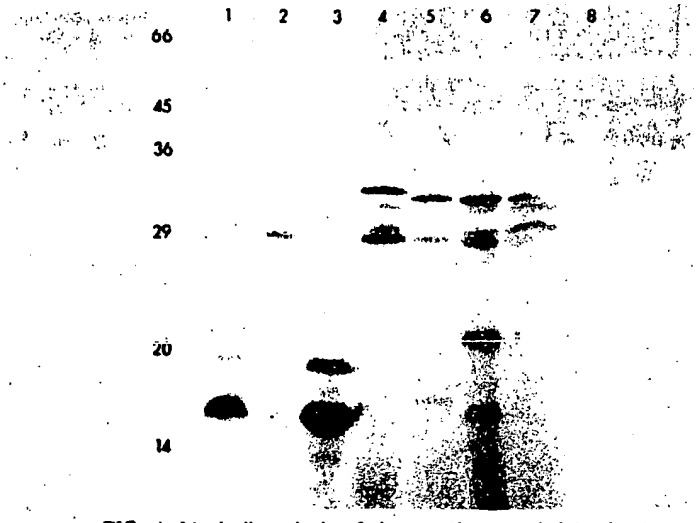


FIG. 4. Maxicell analysis of the proteins encoded in the *lac* region. Labeling of the proteins was as described in the legend to Fig. 3. The plasmids are described in Fig. 2. Shown are lysates of cells carrying the indicated plasmids. Lanes: 1, pBO733; 2, pBO732; 3, pBO697; 4, pBO696; 5, pBO695; 6, pBO694; 7, pMK4; 8, plasmid-free CSR603. The molecular size markers are indicated on the left (in kilodaltons).

(see Materials and Methods). This promoter is negatively regulated by a thermosensitive lambda repressor. When cells carrying the recombinant plasmids were induced by incubation at high temperatures (as described under Materials and Methods), a protein band of 27,000 daltons was detected with the *Eco*RI fragment inserted in the same orientation which had displayed the stronger promoter activity (Fig. 5, lane 3). The band was absent when the *Eco*RI fragment was inserted so that transcription was directed towards phospho-β-galactosidase. It is conceivable that the stronger promoter functions in directing the transcription of the gene for the 27,000-dalton protein. In the opposite orientation, four protein bands were detected corresponding to proteins of 32,000, 20,000 (truncated product; its gene spans the *Eco*RI site), 19,000 (not detected via maxicell analysis and may be an artifact resulting from the plasmid construction), and 17,000 daltons (Fig. 5, lane 5). The genes for these proteins appear to be transcribed in the same direction as the structural genes of the *lac* operon. The presence of the 32,000-dalton protein correlated with repressor activity (Fig. 3 and 4). These results suggest that the repressor gene, along with two other genes of unknown function, is transcribed in the same orientation as the structural genes of the *lac* operon. The genes encoding the 21,500- and 17,000-dalton proteins lay between *lacR* and the known structural genes of the

operon (Fig. 2). It is not known whether these two determinants are part of the staphylococcal *lac* operon.

DISCUSSION

McClatchy and Rosenblum (17) isolated a number of *lac* constitutive mutants and thus inferred the presence of a regulatory locus in the operon. These investigators were not able to localize this locus on the staphylococcal chromosome. However, when Morse et al. (20) examined the genetic linkage between the regulator gene and the structural genes by transduction, their data indicated a tight linkage between *lacG* and the regulatory locus. The data generated in our laboratory confirm the genetic linkage between *lacG* and *lacR*.

We have shown the presence of a regulatory region 2 kb upstream of *lacF* of the *lac* operon. The regulatory region appears to consist of at least two components. The first component is a gene (*lacR*) specifying a polypeptide of approximately 32,000 daltons, which upon expression from a multicopy plasmid confers a noninducible phenotype to the wild-type cell. The most probable explanation for the noninducibility of the cells carrying the cloned repressor gene is the overproduction of the repressor protein. High concentrations of the protein must shut down the operon to such an extent that there is no transport of the inducer (or its phosphorylation upon entry) into the cell. The evidence supporting such hypothesis is that when galactose 6-phosphate (and not galactose) was used as the inducer of the operon, the operon could be induced even in the presence of the plasmids carrying *lacR*. Galactose 6-phosphate presumably enters the cell by the hexose-phosphate transport system, bypassing the need for the enzyme II^{lac}- and factor III^{lac}-mediated entry into the cell. Extracellular galactose would require the enzymes of the *lac* operon for uptake.

The evidence that the 32,000-dalton protein is the *lacR* gene product (i.e., the repressor of the operon) includes correlation of its presence with repressor activity and simultaneous disappearance of this protein as well as loss of repressor activity with clones interrupted at the endonuclease *Pst*I recognition site. Furthermore, because the Tn*551* and EMS-generated constitutive mutants were *trans* recessive to the wild-type allele, the *lacR* product must be a negative regulatory protein.

The second regulatory component of the operon is the locus positioned between the repressor gene and the structural genes of the operon. As is evident from Fig. 2, when this region was introduced into the cell on a multicopy plasmid, constitutive expression of the operon ensued. The multiple copies of this region appear to titrate out the repressor protein, which must have some affinity for this locus. This titration would then result in repressor protein not being available for association with the chromosomal operator to prevent expression of the operon. Therefore, the enzymes encoded by the operon are synthesized in the absence of induction. A different interpretation of these data would be the assumption that the chromosomal fragments carried on plasmids pBO697 and pBO733 encode a positive regulator of the operon and that it is the overproduction of such factor in the cell that results in expression of the operon. Studies are in progress to distinguish between the two possibilities.

A complexity to the staphylococcal *lac* operon is that approximately 2 kb of DNA lies between the presumed operator sequences and the 5′ end of the *lacF* gene. Maxicell data and preliminary DNA sequence analysis suggest that this region encodes proteins with apparent molecular weights of 17,000, 21,500, and 36,000, the latter of which is a truncated version of the actual protein (E. Rosey and G. Stewart, unpublished data). Thus, six structural genes may be genes contained in the *lac* operon. The identities of three of these proteins are unknown at this time.

It is of interest, however, that the genes involved in galactose catabolism (*tag* [5]) have the same intracellular inducer as the *lac* genes. Furthermore, the lactose phosphotransferase system is utilized for galactose uptake (29). One way of interpreting this finding is to assume that both lactose and tagatose operons are under the negative control of the same repressor molecule. The chromosomal location of the *tag* operon is unknown. An attractive hypothesis would be to have the *tag* genes clustered together with the *lac* genes. The sizes of the *tagA*, *tagK*, and *tagI* gene products are 37,000, 52,000, and 100,000 daltons, respectively (2–4). Although we have shown the presence of other proteins encoded within the *lac* region, the apparent molecular weights of these proteins as deduced from SDS-polyacrylamide gels clearly do not correspond to those of *tag* gene products.

Cloning of the repressor gene was the first step toward elucidation of regulatory mechanisms involved in utilization of lactose by *S. aureus*. Experiments are ongoing in our laboratory to define the exact DNA sequence at the insertion site of Tn*551* in our Lac-constitutive mutant, KUS74, and also those sequences which are involved in binding of the repressor molecule. Further mutant analysis and DNA sequencing of the operon should shed more light on the organization and regulation of the *S. aureus lac* operon.

ACKNOWLEDGMENTS

We thank Michael C. Hudson for providing the lactose constitutive mutants.

This work was supported by Public Health Service grant AI-21574 from the National Institutes of Health.

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